Comparative study of Physicochemical and Functional Characteristics in Juices from New Mexican Pomegranate Cultivars (*Punicagranatum*L.) and Wonderful Variety

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Abstract

A study of new pomegranate cultivars was carried out to investigate their potential for industrial use and fresh consumption. Wonderful pomegranate is used worldwide for fresh consumption and industrial purposes. Colour parameters, juice yield, Brix, titratable acidity, organic acids and sugars, vitamin C, and simple phenolics including ellagic acid and punicalagins, total anthocyanins, total soluble phenols, condensable tannins and two antioxidant capacity were measured. Juice colour of new cultivars was similar to that of Wonderful pomegranate. Two new pomegranate cultivars showed higher juice yield (~730 mL/kg arils) compared to Wonderful pomegranate (703.7 mL/kg arils). Citric and malic acids are nearly similar in all pomegranate juices studied. In contrast, cultivars 37-5 and 36-11 showed up to 56% and 10-fold higher vitamin C when compared to Wonderful fresh and processed juice, respectively. Wonderful juices showed higher anthocyanin content compared to juices produced from new cultivars. Of eight simple phenolics identified, ellagic acid showed the higher contents in pomegranate juices. Juice of sample 37-5 showed an outstanding punical agins content (40.87 mg/L), 44% higher than Wonderful pomegranate juice. The TEAC antioxidant activity of three new cultivars juices was higher compared to that of Wonderful juices. This useful information can be employed by the juice processing industry; and it can help select those cultivars for de production of pomegranate juice that is attractive and rich in bioactive compounds.

Keywords

Antioxidant Activity; Juice Quality; Phenolic Composition; Pomegranate; Punicallagins

Introduction

An increasing number of studies have suggested that consumption of fruits can play a protective role in reducing the risk of cancer, diabetes, and cardiovascular diseases; phenolics and vitamins among others components (Kurotani, Nanri, Goto, Mizoue, Noda 2013; Masala, Assedi, Bendinelli, Ermini, Sieri. etc. 2012). Increasing evidence suggests that antioxidant properties are likely to be a contributing factor to the effects of those components in vivo (Kalt and Kushad 2000). Pomegranate is an extraordinarily rich source of vitamin C and phenolic compounds such as ellagic punicalaginas, and anthocyanins. pomegranate juice shows an antioxidant activity three times higher than green tea and red wine (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, Kader 2000; Mena, García-Viguera, Navarro-Rico, Moreno, Batual. etc. 2011).

The juice of "Apaseo", the pomegranate variety produced in Mexico, shows a light red colour which has lower acceptance in contrast to the deep purple colour of the Wonderful pomegranate juice, the industrial standard. In 2011, National Research Institute for Forestry, Agriculture and Animal Husbandry (INIFAP), obtained four new pomegranates cultivars with vermillion colored epidermis, deep red arils and juice color similar to Wonderful, but adapted to subtropical environments with summer rainfalls typical of the Central region of Mexico. Before exploring the potential of commercialization of these

new cultivars, it is necessary to evaluate differences or similitudes on chemical and functional characteristics of their juices when compared to those of Wonderful. Such information is needed to assess their value for fresh consumption and as raw material for the industry. The aim of this study was to compare juice characteristics of four new cultivars with those of Wonderful variety juices. The physicochemical characteristics together with organic acids, total soluble phenols, condensed tannins and anthocyanins were examined. The TEAC and ORAC antioxidant capacities and the characterization and quantification of phenolic acids, flavonols and flavanols by HPLC-DAD were also assessed.

Materials and Methods

Plant Material and Juice Extraction

Ripe fruits of four cultivars labeled as 37-5, 36-11, 37-12, and 33-17 (whose registration is underway) recently developed by INIFAP were harvested from an experimental orchard, in August 2011. For comparisons purposes, fruits of Apaseo pomegranate cultivar were also harvested and Wonderful pomegranate fresh fruits and commercial processed juice were obtained from a local supermarket. Samples of 16 fruits per cultivar were selected randomly and harvested. For each cultivar, the 16 fruits were separated into four groups (n = 4) (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual 2011). Arils were hand-separated from the pith avoiding contamination by components in membranous walls (septum). Juices of each sample were obtained by pressure of arils inside a nylon mesh, and then stored at -80 °C for analysis.

Physicochemical Characteristics

Yield juice was determined based on the juice volume produced by one kilogram of arils. The CIELAB colour parameters "a*", and "b*" were determined using a chromameter (Minolta CR-300, Tokyo, Japan). HUE (= arctang (b*/a*)) and chroma (= (a*2 + b*2)1/2) were calculated. Titratable acidity was determined by AOAC method (2000). Total soluble solids (Brix) were determined using a hand refractometer (Pal-1, ATAGO, Tokyo, Japan). The pH was measured with a pH meter (MI 255 Hanna Inst., Woonsocket, RI, USA).

Organic Acids and Sugars Composition

A HP 1100 series high performance liquid chromatography (HPLC) with a diode array detector (HPLC-DAD) (Agilent Technologies, Inc., Santa Clara,

CA, USA) was used to analyze organic acids and sugars following Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011) method. Organic acids were detected at 210 nm. The same HPLC, elution system, flow rate, and column were used for sugars determination. The detection of sugars was assessed with a refractive index detector (HP 110, G1362A). Pure organic acids and sugars were purchased from Sigma (Distrito Federal, Mexico). Results were expressed as gram per liter of juice (g/L).

Total Soluble Phenols

Total soluble phenols (TSP) were determined according to the Folin-Ciocalteu colorimetric method of Singleton, Orthofer and Lamuela-Raventos (1999). TSP detection was 760 nm. TSP was quantified using gallic acid as a standard and results were expressed as mg of gallic acid equivalents per liter (mg GAE/L).

Condensed Tannins

The vanillin method reported by Desphande, Cheryan and Salunkhe (1986) was used to determine condensed tannins (CT). Detection of CT was carried out at 500 nm and results were reported as mg (+)catechin equivalents per liter (mg (+)CE/L) after comparison with a (+)catechin standard curve.

Total Anthocyanins

Absorbance at 535 nm of juices was recorded in order to calculate total anthocyanins (TAN). TAN was calculated and reported as mg cyanidin 3-glycoside equivalents per litre (mg C3GE/L) as follows (Abdel-Aal and Hucl, 1999):

 $TAN = (A/\epsilon)(vol/1000)$ (MW) (1/sample weight) (106)

Where: TAN = Total anthocyanins (mg C3GE/kg); A = Sample absorbance; ε = Molar absortivity of the cyanidin-3-glicoside (25965 cm⁻¹ M⁻¹); vol = Total volume of anthocyanin extract, and MW = Molecular weight of cyaniding-3-glucoside (449 Da).

Ascorbic and Dehydroascorbic Acids

In order to determine Vitamin C, a HP 1100 Series HPLC-DAD with an Agilent ChemStation Software Plus A.09.xx, and a Zorbaxoctadecylsilane (ODS-C18) reverse-phase column was used. A. The mobile phase consisted of 5 mMcetrimide and 50 mM KH₂PO₄ in methanol/water (1:99, v/v) at pH 4.6. (Corrales-Aguayo, Yahia, Carrillo-López and González-Aguilar, 2008). Ascorbic and dehydroascorbic acids were monitored at 261 and 348 nm, respectively and as mg/L.

Simple Phenols

A HP 1100 series HPLC equipped with a diode array detector was used to determine simple phenols according to García-Falcón, Pérez-Lamela, Martínez-Carballo and Simal-Gándara (2007). A 15 cm × 4.6 mm i.d., 5 µm particle size Zorbax octadecylsilane (ODS-C18) reversed-phase column was used for detection. The identification and quantification of the peaks were carried out from (1) the retention times and (2) the spectra derived from DAD in comparison with those from authentic standards. Gallic, protocatechuic, 4hydroxybenzoic, vanillic, chlorogenic, caffeic, syringic, coumaric, ferulic, benzoic and salicylic acids were detected at 280 nm. (+) Catechin, epicatechin (EC), and epigallocatechin gallate (EGCg) were also detected at 280 nm; while ellagic acid, quercetin, rutin and punicalagins were detected at 260 nm.

Antioxidant Activities

The Trolox equivalent antioxidant capacity (TEAC) was assessed by ABTS⁺ radical cation at 734 nm monitored for 6 min. The decrease in absorption after addition of juice was used to calculate the TEAC value compared with a standard curve of TROLOX. Results were expressed in terms of mmol Trolox equivalent per L of sample (mmol TE/L). The oxygen radical absorbance capacity assay (ORAC) of samples carried out according to the procedure reported by Ou, Hampsh-Woodill and Prior (2001) was expressed as milimoles of Trolox equivalents per liter (mmol TE/L).

Statistical Analysis

All data were reported as means ± standard deviations (n = 4). Statistical analysis was performed using the JMP.5.0.1 software (a business unit of SAS, 1989–2003 SAS Institute Inc., NC, USA). Differences among means were tested for significance by ANOVA procedures and Tukey's test, using a level of significance of 0.05.

Results and Discussion

CIELAB Parameters

Colour is a crucial property of a product, since it represents the first contact between the consumer and the product and hence affecting the sale directly (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011). CIE L* values of pomegranate revealed that juices of new cultivars 37-5, 36-11 and 37-12 showed similar darkness to Wonderful pomegranate juice (Table 1). The commercial juice showed higher level of L* (lighter colour) compared to Wonderful pomegranate

juice, probably due to the addition of apple juice, as stated on the label. Redness of cultivar 37-5 juice was similar to that of the Wonderful variety juice; meanwhile, yellowness showed lower differences among juices with the exception of commercial juice. Level of chroma values (C*) of 37-5, 36-11 and 37-12 cultivars was almost similar to that of the Wonderful juice. On the other hand, CIELAB levels reported here for Wonderful juice were in the range reported by Mena, García-Viguera, Navarro-Rico, Moreno, Bartual (2011) with the exception of C*.

Juice Yield

Juice yield is an essential parameter for the juice industry since many processing decisions will be taken based on this value (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011). The range of juice yield obtained varied, between 611.2 and 732.6 mL/kg of arils (Table 1). The highest juice yield was showed by cultivar 33-17 which yielded 4.1% (~ 30 Ml) more than Wonderful variety. This result is of interest since slight differences in juice yield might be translated into bigger profits for the food industry when processing at large scale. Juice yield reported here were higher to those reported by Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011).

Physicochemical Characteristics

Total soluble solids (TSS) of juices of pomegranate cultivars assessed in the present work were similar to TSS content of Wonderful juice (Table 1). TSS levels of Wonderful juice reported here, were similar to those reported by Fadavi, Barzegar, Asisi and Bayat(2005) and Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011). Titratable acidity (TA) values varied from 2.4 (cultivar 33-17) to 15.8 g/L (Wonderful variety). TA of Mexican pomegranate juices was 1.6 (cultivar 37-12) to 6.6-fold (cultivar 33-17) lower than TA detected in Wonderful juice which showed the highest value. TA levels reported here were similar to those reported for Spanish pomegranates, Israeli, Turkish and Iranian fruits (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011; Bar-Ilan, Holland, Aviram. etc. 2007; Poyrazodlu, Gökmen and Artik, 2002; Fadavi, Barzegar, Asisiand Bayat, 2005). Maturity index (TSS/TA) oscillated between 11.3 (Wonderful variety) and 52.4 (cultivar 33-17) (Table 2). Maturity index reported here for Wonderful pomegranate was in the range reported by Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011). pH of pomegranate juices (2.9 - 3.6) including Wonderful juices was in the range of pH reported for pomegranate elsewhere (Fadavi, Barzegar, Asisi and Bayat, 2005; Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011).

The major organic acids detected in pomegranate cultivars were citric acid (CA) and L-malic acid (Table 2). Oxalic, quinic, succinic and tartaric acids were also detected, as reported by Poyrazodlu, Gökmenand Artik (2002) for pomegranate. In general CA contents of new cultivars and the commercial Apaseo variety were two-fold the content of malic acid, which is more likely to that observed in Wonderful varieties than in Spanish juices as reported by Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011). For example, CA contents of cultivars 37-5, 36-11 and 37-12 (2.49 - 3.58 g/L) juices were similar to those of Wonderful juices reported here and by Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. ect. (2011) and Poyrazodlu, Gökmen and Artik (2002). However, CA contents of cultivar 33-17 and Apaseo variety were similar to those of Spanish cultivars already reported (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011).

Regarding sugar contents in pomegranate juices,

glucose values ranged from 73.2 g/L (Apaseo) to 89.4 g/L (Wonderful); meanwhile, fructose ranged from 73.2 g/L (commercial juice) to 90.1 g/L (cultivar 33-17) (Table 2).Glucose content in all juices was higher than fructose levels (G/F ratios > 1) with the exception of line 33-17 and Apaseo variety juice. Pomegranate juices with G/F ratios less than 1.0 are not in agreement with that for G/F ratio established by the Association of the Industry of Juices and Nectars of the European Union (AIJN, 2008). Results of G/F ratios reported here were similar to those reported by Fadavi, Barzegar, Asisi and Bayat (2005).

Vitamin C

All samples did not exceed 130 mg/L of vitamin C with the exception of cultivar 36-11 (Table 3). Therefore, the juice of this line could be most susceptible to undesirable browning and low stability of anthocyanins; however, this is not a problem in intensely dark coloured juices. Levels of Vitamin C reported here were consistent with those previously reported for Spanish, Israeli and Turkish cultivars (Fadavi, Barzegar, Asisi and Bayat, 2005; Poyrazodlu, Gökmen and Artik, 2002).

TABLE 1 CIELAB L*, A* AND B*PARAMETERS, HUE (H), CHROMA (C*), JUICE YIELD, TOTAL SOLUBLE SOLIDS (TSS), TITRATABLE ACIDITY (TA), TSS/TA RATIO AND PH, OF POMEGRANATE JUICES.^A

Sample	L*	a*	b*	Н	C*	Juice yield (ml/kg arils)	TSS (Brix, 25 °C)	TA (g CA ^b /L)	Ratio (TSS/TA)	рН
37-5	6.2±0.17 f	1.9±0.07 e	0.94±0.02 de	26.8±0.38 b	2.1 ±0.07 f	730.2±15 a	17.7 ± 0.62 a	9.3±0.04 d	19.1±0.7 c	2.9±0.04 c
36-11	7.4±0.24 d	3.3±0.27 d	0.97 ±0.03 d	16.6±1.36 e	3.4±0.28 d	611.2±42 c	17.3 ± 1.03 a	4.1±0.02 e	43.3 ±2.6 b	3.6±0.1 a
37-12	7.2±0.33 e	$1.7 \pm 0.05 \; \mathrm{f}$	2.21 ±0.05 b	34.8±2.38 a	2.8±0.06 e	$612.2 \pm 26 \mathrm{c}$	17.7 ± 0.64 a	9.7±0.02 c	18.2±0.4 d	$3.1 \pm 0.3 b$
33-17	8.6±0.32 c	4.6 ±0.29 c	1.70±0.05 c	20.3±0.86 d	4.9±0.32 c	732.6 ± 16 a	17.1 ± 0.31 a	2.4±0.02 g	$52.4 \pm 3.2 a$	3.0±0.1 b
Apaseo	11.2±0.71 a	6.4±0.14 b	$0.81 \pm 0.09 \text{ f}$	7.3±0.67 f	6.4±0.14 b	$661.1 \pm 37 \text{ b}$	15.2 ± 0.18 c	$3.3 \pm 0.02 \text{ f}$	45.3±1.7 b	2.9±0.1 bc
Wonderful	7.6±0.10 d	2.1±0.04 e	0.84±0.07 ef	21.9±1.29 d	2.2 ±0.06 f	$703.7 \pm 21 \text{ b}$	17.8 ± 0.58 a	15.8±0.03 a	$11.3\pm0.6~\mathrm{f}$	2.9±0.03 c
CJ^c	9.8±0.10 b	15.1±0.21a	6.92±0.34 a	24.5 ± 0.42 c	16.6±0.2 a		15.9 ± 0.07 b	10.3±0.01 b	15.5 ± 0.3 e	3.1±0.02 b

^aMeans in the same column with a common letter are not significantly different (p<0.05). ^bCitric acid. ^cCommercial juice.

TABLE 2 ORGANIC ACIDS AND SUGAR COMPOSITION (G/L) AND RATIO GLUCOSE/FRUCTOSE (G/F) OF POMEGRANATE JUICES.^A

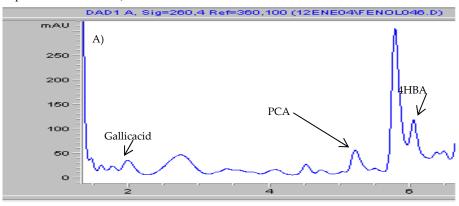
	37-5	36-11	37-12	33-17	Apaseo	Wonderful	СJь
Organic acids							
Citric	3.58 ± 0.31 a	$3.06 \pm 0.11 \text{ b}$	2.49 ± 0.26 c	$0.88 \pm 0.04 d$	$0.94 \pm 0.09 \text{ d}$	3.29 ± 0.19 ab	2.41 ± 0.10 c
Malic	1.11 ± 0.08 c	1.23 ± 0.06 b	1.16 ± 0.07 bc	1.46 ± 0.13 a	1.51 ± 0.18 a	1.53 ± 0.12 a	$0.79 \pm 0.0.5 d$
Oxalic	0.27 ± 0.01 d	0.33 ± 0.08 c	$0.22 \pm 0.0 \ 2e$	0.48 ± 0.05 b	0.32 ± 0.06 cd	0.43 ± 0.06 bc	0.70 ± 0.06 a
Quinic	0.18 ± 0.02 a	0.12 ± 0.01 b	ND	ND	0.12 ± 0.03 b	0.09 ± 0.00 c	0.15 ± 0.04 ab
Succinic	ND	ND	0.20 ± 0.0 a	0.19 ± 0.02 b	0.16 ± 0.03 b	0.14 ± 0.01 c	ND
Tartaric	0.60 ± 0.08 c	0.93 ± 0.02 a	$0.78 \pm 0.06 \text{ b}$	0.56 ± 0.03 c	0.92 ± 0.08 a	0.85 ± 0.07 ab	ND
Sugars							
Glucose	$85.5 \pm 2 a$	$89.3 \pm 4 a$	$86.5 \pm 3 \text{ a}$	$80.1 \pm 1 \text{ b}$	$73.2 \pm 2 \text{ c}$	89.4 ± 2 a	$81.2 \pm 3 \text{ b}$
Fructose	$76.2 \pm 1 \text{ c}$	$77.5 \pm 3 \text{ bc}$	$84.8 \pm 4 \text{ ab}$	90.1 ± 3 a	75.9 ± 1 c	$89.0 \pm 2 a$	$73.2 \pm 6 \text{ c}$
G/F Ratio	1.12	1.15	1.02	0.89	0.96	1.00	1.11

 $^{^{\}mathrm{a}}$ Means in the same row with a common letter are not significantly different (p<0.05). $^{\mathrm{b}}$ Commercial juice.

TABLE 3 VITAMIN C, TOTAL SOLUBLE PHENOLS (TSP), CONDENSED TANNINS (CT), AND TOTAL ANTHOCYANINS (TAN) OF POMEGRANATE JUICES.^A

Sample	V	itamin C (mg/L)		TSP (mg GAE ^b /L)	CT (mg (+)CE ^c /L)	TAN (mg C3GEd/L)	
	Ascorbic acid	Dehidroascorbic acid	Total				
37-5	103.0 ± 4 b	15.4 ± 3.1 a	118.4	3200 ± 28 c	237 ± 17 d	617 ± 44 b	
36-11	136.1 ± 9 a	$12.0 \pm 1.0 \text{ ab}$	148.1	$3519 \pm 76 \text{ b}$	$285 \pm 17 \text{ c}$	$398 \pm 30 \text{ d}$	
37-12	$86.0 \pm 6 \text{ cd}$	$10.3 \pm 0.9 \text{ b}$	96.3	$3273 \pm 41 \text{ c}$	$648 \pm 25 \text{ a}$	$812 \pm 50 \text{ a}$	
33-17	$83.3 \pm 6 \text{ cd}$	1.6 ± 0.16 e	84.9	2112 ± 57 e	$228 \pm 12 d$	$623 \pm 52 \text{ b}$	
Apaseo	$75.4 \pm 6 d$	$2.6 \pm 0.10 d$	78.0	$2523 \pm 29 d$	$119 \pm 48 \text{ e}$	$469 \pm 44 \text{ c}$	
Wonderful	$88.2 \pm 3 \text{ c}$	$6.7 \pm 26.2 \text{ c}$	94.9	$3806 \pm 43 \text{ a}$	$535 \pm 39 \text{ b}$	$888 \pm 20 \text{ a}$	
CJe	$12.9 \pm 1.0 e$	ND	12.9	$3797 \pm 62 a$	296 ± 19 c	$895 \pm 61 \text{ a}$	

^aMeans in the same column with a common letter are not significantly different (p<0.05). ^bGallic acid equivalents. ^c(+)Catechin equivalents. ^dCynidin 3-glucoside equivalents. ^eComercial Juice



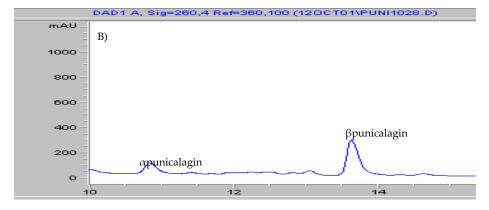


FIG. 1 CHROMATOGRAPHIC PROFILE OF A) PHENOLIC ACIDS IDENTIFIED IN JUICE OF CULTIVAR 36-11 AND B) PUNICALAGINS DETECTED IN JUICE OF WONDERFUL VARIETY. PCA = PROTOCATECHUIC ACID; 4HBA = 4-HYDROXIBENZOIC ACID.

TABLE 4 SIMPLE PHENOLS (MG/L) COMPOSITION OF POMEGRANATE JUICES.^A

Sample	Benzoic acid	Ellagic acid	Gallic acid	4HBA	PCA ^b	Siringic acid	EGCg ^c	Punicalagin	Total
37-5	2.37 ± 0.35 e	12.8 ± 0.52 a	16.5±1.1 c	11.2±0.23 c	18.9 ± 1.12 c	16.9 ± 1.7 b	8.13 ± 1.64 a	40.87 ± 0.69 a	127.69
36-11	$4.05 \pm 0.15 \text{ b}$	$10.8 \pm 0.46 \text{ b}$	12.6±1.5 d	10.0±0.49 d	17.6 ± 0.92 c	$14.9 \pm 0.95 d$	4.56 ± 0.56 c	$3.77 \pm 0.06 d$	78.28
37-12	4.67 ± 0.26 a	11.2 ± 0.23 b	27.5±1.7 a	$27.4 \pm 2.5 a$	34.9 ± 1.97 a	24.1 ± 1.15 a	$9.23 \pm 1.2 \ a$	2.37 ± 0.50 e	141.37
33-17	4.43 ± 0.24 ab	10.2 ± 0.39 b	14.6±1.8 d	$14.5 \pm 1.8 \text{ b}$	$18.0 \pm 2.55 d$	$18.0 \pm 0.90 \text{ b}$	$5.83 \pm 0.22 \text{ b}$	2.41 ± 0.07 e	87.97
Apaseo	3.77 ± 0.19 c	$10.8 \pm 0.18 \text{ b}$	18.1±1.9 bc	$24.3 \pm 1.4 \text{ a}$	32.0 ± 1.16 a	13.4 ± 1.92 c	5.43 ± 0.34 bc	6.99 ± 0.26 c	114.79
Wonderful	$3.02 \pm 0.39 d$	9.58 ± 0.20 c	19.2±1.4 b	7.01 ±0.22 f	24.4 ± 0.89 b	17.5 ± 0.34 b	$1.07 \pm 0.21 d$	28.36 ± 1.64 b	110.14
CJ^d	3.39 ± 0.25 cd	9.59 ± 0.28 c	5.2 ± 0.64 e	9.31±1.22 e	23.6 ± 0.04 b	17.0 ± 0.42 b	$1.23 \pm 0.19 d$	18.43 ± 0.91 c	87.74

 ${}^a\!M\!eans \ in \ the \ same \ column \ with \ a \ common \ \ letter \ are \ not \ significantly \ different \ (p<0.05). \ {}^b\!Protochatecuic \ acid. \ {}^c\!Epigallocateching \ all \$

Phenolic Compounds

Pomegranate juices of the new cultivars and Apaseo variety showed lower total soluble phenols (TSP)

compared to contents of Wonderful and commercial juices (Table 3). Similar results were observed in condensed tannins (CT) with exception of cultivar 37-12 juice which showed 21% higher contents when

compared to Wonderful juices. Contents of TSP reported here for Mexican pomegranate and Wonderful varieties were similar to TSP levels reported for Spanish cultivars juices (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011) and higher to Wonderful varieties juices from California (2117 mg/L) (Gil, Tomás-Barberán, Hess-Pierce, Halcroft and Kader 2000).

Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011) who reported that Wonderful pomegranate juices were by far higher in TSP contents (up to ~4500 mg/L) compared to those of Spanish varieties juices, as also demonstrated here for Mexican varieties juices.

On the other hand, total anthocyanin (TAN) levels detected in cultivar 37-12 juice (812 mg C3GE/L) was similar to those detected in Wonderful variety juice (888 mg C3GE/l) and in commercial juice (895 mg C3GE/L) (Table 3). The lower TAN content of cultivar 36-11 and Apaseo variety juices mirrored the colour of its juice (light red). TAN levels reported here for Mexican cultivars juices were higher compared to TAN contents of Spanish and Tunisian cultivars juices (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011; El Karr, Ferchichi, Attia and Bouajila 2011).

Simple Phenols

The HPLC chromatograms of simple phenols detected in cultivar 36-11 and punicalagins detected in Wonderful juice are shown in Figure 1. Benzoic, ellagic, 4-hydroxybenzoic, siringic and protocatechuic acids were identified together with the flavanol epigallocatechin gallate (EGCg), and the ellagitanin punicalagins (Table 4). Of these compounds, benzoic acid and EGCg are

found to be the minor components in all juices, together with punicalagin in cultivars 36-11, 37-12, 33-17 and Apaseo variety. It is important to indicate that cultivar 37-5 shows an outstanding content of punicalagins, which represents 32% of the total simple phenolics detected. Higher variations in punicalagins content reported here are similar to those reported by Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011) for Spanish cultivars and Wonderful pomegranates. Other peaks were detected, but they did not match any of the standards used in the present work. Such peaks could be proanthocyanidins with different degree of polymerization (Mena, García-Viguera, Navarro-Rico, Moreno, Bartual. etc. 2011), 3-O-caffeoylquinic acid, cyanidins, unknown quercetin derivatives or hydroxycinnamic acid (Fisher, Carle and Kammerer, 2011) reported in pomegranate. This is the first report about the presence of EGCg, and other simple phenolic in pomegranate.

Antioxidant Activities

In general, higher TEAC values for pomegranate juices than ORAC values are observed (Figure 2). These TEAC and ORAC activities are correlated to the TSP and vitamin C of pomegranate juices with exception of ascorbic acid levels of Wonderful varieties and commercial juices. A higher correlation has been observed between TSP and TEAC levels (R = 0.73, p<0.05). Not being underestimated in the TEAC antioxidant activity is the contribution of the phenolic acids and TAN; however, contrary to our results, the ellagic acid and TAN show a positive correlation (R = 0.85, p<0.001; 0.72 p<0.001) with Mena García-Viguera, Navarro-Rico, Moreno, Bartual. etc. (2011).

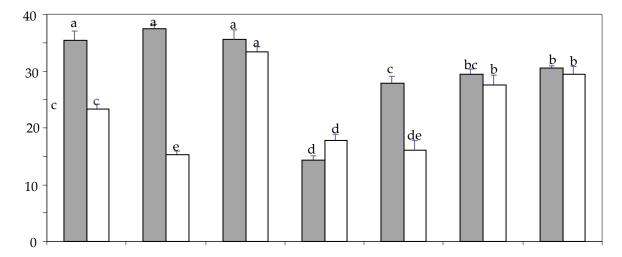


FIG. 2. TEAC AND ORAC ACTIVITIES (mmol TROLOX EQUIVALENT/L) OF POMEGRANATE JUICES. MEANS BETWEEN THE SAME ANTIOXIDANT ACTIVITY WITH A COMMON LETTER ARE NOT SIGNIFICANTLY DIFFERENT (P < 0.05, TUKEY'S)

Conclusions

In the present study, we have compared and characterized the quality and functional parameters, and antioxidant activity of the juices of new developed Mexican pomegranate cultivars, as well as commercial juice obtained from Wonderful pomegranates widely commercialized in Mexico and in the rest of the world. Mexico is promoting the production of pomegranate based on varieties adapted to our agro climatic conditions but showing similar quality to that already available in the world market. Little variation among new cultivars juices was detected considering all measured variables; however, the outstanding content of punical agin of cultivar 37-5 makes it more valuable from the nutraceutical point of view. On the other hand, cultivar 33-12 showed juice yield higher to Wonderful, lower acidity, and richer in bioactive compounds than this cultivar. The outstanding content of vitamin C of the juice of cultivars 37-5, 36-11 and 37-12 compared to Wonderful is worthwhile to note from the nutritional point of view. On the other hand, the antioxidant activities of cultivars 37-5, 36-11 and 37-12 juices were higher compared to those of Wonderful variety juice. Overall, pomegranates juices of Mexican new cultivars showed a significant higher antioxidant capacity than most commonly consumed fruits and juices. This information can provide the industry with a useful approach to select Mexican pomegranate cultivars well suited for juice elaboration and a starting point for the development of appetizing pomegranate juices with higher levels of bioactive compounds.

REFERENCES

- Abdel Aal, E.S.M.andHucl, P. J.:Rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats.Cereal Chem. 76(3), 350-354 (1999).
- AIJN. Association of the Industry of Juices and Nectars of the European Union, Reference Guideline for Pomegranate Juice. Code of Practice for Evaluation of Fruit and Vegetable Juices 6.21,2008.
- AOAC. Official methods. Arlington, VA: Association of Official Analytical Chemists International, 2000.
- Corrales-Aguayo, R. D.,Elhadi M. Y., Carrillo-López, A. andGonzález-Aguilar, G. Correlation between some nutritional components and the total antioxidant capacity measured with six different assays in eight horticultural

- crops. J.Agric. Food Chem. 56, 10498-10504(2008).
- Desphande, S. S., Cheryan M. and Salunkhe D.K. Tannin analysis of food products.CRC Crit. Rev. Food Sci. Nutr. 24, 401–449 (1986).
- El Karr, C.,Ferchichi, A., Attia F. and Bouajila, J. Pomegranate (*Punicagranatum*) juices: chemical composition, micronutrient cations, and antioxidant capacity. J. Food Sci. 76(6), 795-800 (2011).
- Fadavi, A., Barzegar, M., Azizi, M.H. and Bayat, M. Note. Physicochemical composition of ten pomegranate cultivars (Punicagranatum L.) grown in Iran. Food Sci. Technol. Int. 11, 113-119 (2005).
- Fisher, U. A., Carle, R. and Kammerer, D. R. Identification and quantification of phenolic compounds sfrompomegranate (Punicagranatum L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-EIS/MS. Food Chem. 127, 807-821 (2011).
- García-Falcón, M. S, Pérez-Lamela, C., Martínez-Carballo, E., and Simal-Gándara J. Determination of phenolic compounds in wines: influence of bottle storage of young red wines on their evolution. Food Chem. 105, 248-259 (2007).
- Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M. and KaderA. A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. FoodChem. 48,4581–4589 (2000).
- Kalt, W. and Kushad, M.M. The role of oxidative stress and antioxidants in plant and human health. HortScience 35(4), 572 (2000).
- Kurotani, K., Nanri, A., Goto, A., Mizoue, T. Noda, N., Kato, M., Inoue M. and Tsugane, S. Vegetable and fruit intake and risk of type 2 diabetes: Japan Public Health Centerbased Prospective Study. British J. Nutr. 9,709-717 (2013).
- Masala, G., Assedi, M., Bendinelli, B., Ermini, I., Sieri, S., Grioni, S., Sacerdote, C., Ricceri, F., Panico, S., Mattiello, A., Tumino, R., Giurdenella, M. C., Berrino, F., Saieva, C. and Palli, D. Fruit and vegetables consumption and breast cancer risk: the EPIC Italy study. Breast Cáncer Res. Treat. 132(3), 1127-1136 (2012).
- Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D. and Marti, N. Phytochemical characterisation for industrial use of pomegranate (*Punicagranatum* L.) cultivars grown in Spain. J. Agric.

Food Chem. 91(10), 1893-1906 (2011).

Ou, B., Hampsh-Woodill, M. and Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as a fluorescent probe. J. Agric. Food Chem. 49, 4619–4626 (2001).

Ozgen, M., Durac, C., Serce S. and Kaya, C. Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chem. 111, 703-706 (2008).

Poyrazodlu, E., Gökmen, V. and Artik, N. Organic acids and phenolic compounds in pomegranate (*Punicagranatum* L) grown in Turkey. J. Food Comp. Anal. 15, 567-575 (2002).

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by jeans of the Folin–Ciocalteu reagent. Meth. Enzymology. 299, 152–178 (1999).

Bar-Ilan, I., Holland, D., Aviram, M. and Amir, R. Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions .J Agric. Food Chem. 55(23). 95, 59-70 (2007).

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